PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P10418PC	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DK 03/00383	International filing date *(day/month/year)* 11.06.2003	Priority date *(day/month/year)* 11.06.2002
International Patent Classification (IPC) or both national classification and IPC G01N15/12		
Applicant CHEMPAQ A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.01.2004	26.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Koch, A Telephone No. +31 70 340-3828

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00383

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21 as originally filed

Claims, Numbers

1-18 filed with telefax on 10.08.2004

Drawings, Sheets

1/9-9/9 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18
	No:	Claims	
Inventive step (IS)	Yes:	Claims	7,8
	No:	Claims	1-6, 9-18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D2: US-A-5 231 005 (COULTER WALLACE H ET AL) 27 July 1993 (1993-07-27)
D3: US-B-6 387 328 B1 (BERNDTSSON INGEMAR) 14 May 2002 (2002-05-14)
D4: WO 93/01306 A (GOUMENIOUK ALEXANDER P ;RICHARDS BRIAN G (CA)) 21 January 1993 (1993-01-21)
D5: US-A-5 501 982 (RUTNARAK SANGVORN ET AL) 26 March 1996 (1996-03-26)
D6: US-A-5 731 206 (LEDIS STEPHEN L ET AL) 24 March 1998 (1998-03-24)

1. The closest prior art of amended claim 1 is seen in the document D3 disclosing a cartridge for blood testing.

1.1 Regarding claim 1, D3 discloses:
A cartridge for counting and discriminating a plurality of types of blood cells in a blood sample (col. 1, l. 11-14; col. 3, l. 28-31 of D3) in one counting operation (col. 3, l. 28-31; col. 4, l. 13-14), comprising a housing with characterizing particles suspended in a liquid sample (col. 3, l. 28-31; col. 3, l. 39-48),
connectors for operational connection to and disconnection from connectors of a docking station for establishment of electrical and fluid connections when the cartridge is received in the docking station (col. 4, l. 4-15; col. 6, l. 7-22 of D3),
a first mixing chamber (col. 4, l. 57-61),
first cell characterization means for characterizing cells passing through the first orifice (col. 4, l. 4-15)
a bore in the outer surface of the housing for entrance of the blood sample (col. 4, l. 24-33, "channel 54"), communicating with
a first sampling member positioned in the housing for sampling the blood sample and having a first cavity for receiving and holding the blood sample (col. 3, l. 49-55, figs. 2-4 of D3; first cavity: "through channel 53"), the member being movably positioned in relation to the housing in such a way that, in a first position, the first cavity is in communication with the bore for entrance of the blood sample into the first cavity (col. 3, l. 56-62 of D3), and, in a second position, the first liquid storage chamber ("intake channel 54", col. 3, l. 56-62; col. 4, l. 28-33) communicates through the first cavity with the first mixing member so that the blood sample can be flushed with discharged liquid from the first liquid storage chamber into the first

mixing chamber (col. 3, l. 63-67; col. 4, l. 55-65),
characterized in that the cartridge further comprises
a first collection chamber separated by a wall from the first mixing chamber, the wall containing a first orifice for the passage of the particles between the first mixing chamber and the first collection chamber (col. 5, l. 18-24), and in that the first particle characterization means is adapted for characterization of the particles passing through the first orifice (col. 5, l. 18-30).

1.2 Claim 1 specifies over document D3: the technical feature of
- a first liquid storage chamber for holding a lysing reagent with a lysing capability sufficient for lysing of erythrocytes while maintaining counting ability of other blood cell types.

1.3 The technical problem solved by this technical feature over the closest prior art document D3 is:
- counting other blood cell types than erythrocytes.

1.4 The skilled person seeking a solution to the technical problem mentioned under section 1.3 of this Report would also come across document D5 describing another disposable cartridge for use with an analytical instrument for blood cell analysis. Document D5 discloses the technical feature of claim 1 over D3, c.f. col. 3, l. 27-30 and fig. 1; col. 7, l. 25-29, figs. 3 and 4 of D5). D5 also discloses the problem under section 1.3 of this Report , c.f. col. 3, l. 28-31 of D5. The skilled person seeking a solution to this technical problem would use the teaching of D5 to modify the cartridge of D3 and thus arrive at a cartridge according to claim 1, without an inventive step being involved. Therefore claim 1 does not comply with Articles 33(1) and (3) PCT.

2. Dependent claims 2-6 and 9-18 do not fulfill the requirements of Articles 33(1) and (3) PCT, for the following reasons:

2.1 Concerning claims 2 and 3: The document D6 which refers to a kit of a lytic reagent system and a lytic reagent composition anticipates a lysing reagent containing saponin for lysing erythrocytes and analysing other blood cell types (col. 7, l. 45-63 of D6). The person skilled in blood analysis techniques would know that saponin can be used in a surfactant. All the technical features of claims 2 and 3 can be assumed to be general knowledge of the skilled person.

2.2 The technical features of claim 4 beyond those of claim 1 are already known from D5, for the same or a similar technical purpose (c.f. col. 3, l. 30/31).

2.3 The technical features of claim 5 beyond those of the claims to which it refers are already known from D3, for the same or a similar technical purpose.

2.4 The technical features of claim 6 beyond those of the claims to which it refers are already known from D6 (col. 7, l. 45-63 of D6), for the sample or a similar technical purpose.

2.5 The technical features of claim 9 beyond those of the claims to which it refers are known from D5, for the same or a similar technical purpose (col. 7, l. 41-54 of D5).

2.6 The technical features of claim 10 beyond those of the claims to which it refers are known from D5 (col. 7, l. 29-31) and are part of the knowledge of the skilled person.

2.7 Concerning claim 11: D2 which discloses a method and apparatus for automatic analysis and counting of different types of blood cells describes the use of a magnetic mixing member in a mixing chamber (col. 7, l. 64-col. 8, l. 6; col. 10, l. 33-36 of D2). It would be obvious to the skilled person to apply a magnetic mixing member also in the mixing chamber mentioned in the application.

2.8 The features of claims 12 and 13 beyond those of the claims to which they refer are known from D3 (col. 4, l. 10-15), for the same or a similar technical purpose.

2.9 The features of claims 14-18 which all refer to the dimensions of the orifice are considered general technical knowledge of the person killed in the field of blood analysis by Coulter counter techniques. Since the rough dimensions of the types of blood cells which are to be analyzed are all known, it is evident for the skilled person to adapt the dimensions of the orifice accordingly.

3. Remaining claims 7 and 8 seem to comprise features ("second collection chamber", "second cell characterization means") which are not anticipated by any of the prior art documents, and which seem to be novel and inventive in the sense of Articles 33(1)-(3) PCT.

CLAIMS

1. A cartridge for counting and discriminating a plurality of types of blood cells in a blood sample in one counting operation, comprising a housing with

a first mixing chamber,

first cell characterization means for characterizing cells passing through the first orifice,

a bore in the outer surface of the housing for entrance of the blood sample, communicating with

a first sampling member positioned in the housing for sampling the blood sample and having a first cavity for receiving and holding the blood sample, the member being movably positioned in relation to the housing in such a way that, in a first position, the first cavity is in communication with the bore for entrance of the blood sample into the first cavity, and, in a second position, the first liquid storage chamber communicates through the first cavity with the first mixing chamber so that the blood sample can be flushed with discharged liquid from the first liquid storage chamber into the first mixing chamber

c h a r a c t e r i z e d in that the cartridge further comprises

a first liquid storage chamber for holding a lysing reagent with a lysing capability sufficient for lysing of erythrocytes while maintaining counting ability of other blood cell types, and

a first collection chamber separated by a wall from the first mixing chamber, the wall containing a first orifice for the passage of the cells between the first mixing chamber and the first collection chamber, and in that

the first cell characterization means is adapted for characterization of the particles passing through the first orifice.

2. A cartridge according to claim 1, wherein the lysing reagent contains a surfactant.

3. A cartridge according to claim 1 or 2, wherein the surfactant comprises a saponin.

4. A cartridge according to claim 1, wherein the lysing reagent comprises a quaternary ammonium salt.

5. A cartridge according to any of the preceding claims, wherein cells of the other cell types are reduced in size and the concentration is determined by counting a representative fraction of the respective cells.

6. A cartridge according to any of the preceding claims, wherein the other cell types include sub-populations of leukocytes, such as lymphocytes, monocytes and granulocytes, which are selectively reduced in size by the lysing reagent and can be counted in a cell counter.

7. A cartridge according to any of the preceding claims, further comprising

a second mixing chamber and a second collection chamber separated by a second wall containing a second orifice for the passage of the cells between the second mixing chamber and the second collection chamber,

second cell characterization means for characterizing cells passing through the second orifice, and wherein

in the second position, the first cavity is in communication with the first mixing chamber for entrance of liquid from the first mixing chamber into the first cavity, and, in a third position, the first cavity is in communication with the second mixing chamber for discharge of the liquid in the first cavity into the second mixing chamber.

8. A cartridge according to any of claims 1-6, further comprising

a second mixing chamber and a second collection chamber separated by a second wall containing a second orifice for the passage of the cells between the second mixing chamber and the second collection chamber,

second cell characterization means for characterizing cells passing through the second orifice, and

a second sampling member positioned in the housing for sampling a small and precise volume of liquid from the first mixing chamber and having a second cavity for receiving and holding the sampled liquid, the member being movably positioned in relation to the housing in such a way that, in a first position, the second cavity is in communication with the first mixing chamber for entrance of liquid from the first mixing chamber into the first cavity, and, in a second position, the second cavity is in communication with the second mixing chamber for discharge of the sampled liquid in the second cavity into the second mixing chamber.

9. A cartridge according to any of the preceding claims, further comprising a reagent chamber positioned adjacent to the first mixing chamber for holding a reagent to be entered into the first mixing chamber.

10. A cartridge according to claim 9, further comprising a breakable seal separating the reagent chamber from the first mixing chamber.

11. A cartridge according to any of the preceding claims, wherein a mixing member is positioned in at least one of the mixing chambers.

12. A cartridge according to any of the preceding claims, further comprising a sensor for characterization of the liquid.

13. A cartridge according to claim 12, wherein the sensor for characterization of the liquid is adapted for spectrophotometric characterization of the liquid.

14. A cartridge according to any of the preceding claims, wherein the orifice has a diameter in the range from 30 μm to 100 μm.

15. A cartridge according to claim 14, wherein the orifice has a diameter in the range from 35 μm to 50 μm.

16. A cartridge according to claim 15, wherein the orifice has a diameter in the range from 30 μm to 45 μm.

17. A cartridge according to claim 16, wherein the orifice has a diameter in the range from 35 μm to 40 μm.

18. A cartridge according to claim 17, wherein the orifice has a diameter substantially equal to 40 μm.